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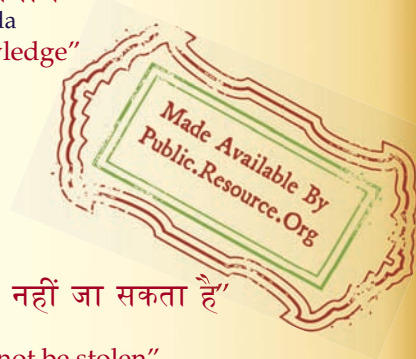
IS 5887-7 (1999): Methods for Detection of Bacteria Responsible for Food Poisoning, Part 7: General Guidance on Methods for Isolation and Identification of Shigella [FAD 15: Food Hygiene, Safety Management and Other Systems]



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भारतीय मानक

खाद्य विषाक्तता उत्पन्न करने वाले जीवाणुओं की
संसूचन पद्धतियाँ

भाग 7 शिगेला का विलगन करने और पहचान के लिए पद्धतियों के सामान्य
मार्गदर्शी सिद्धान्त

Indian Standard

METHODS FOR DETECTION OF BACTERIA
RESPONSIBLE FOR FOOD POISONING

PART 7 GENERAL GUIDANCE ON METHODS FOR ISOLATION AND
IDENTIFICATION OF *SHIGELLA*

ICS 07.100.30

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BUREAU OF INDIAN STANDARDS
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NEW DELHI 110002

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Food Microbiology Sectional Committee had been approved by the Food and Agriculture Division Council.

Several micro-organisms contaminating food give rise to clinical symptoms, which include abdominal pain, nausea, vomiting, diarrhoea and sometimes pyrexia. Poisoning through food is characterized by the explosive nature with which the symptoms occur in otherwise healthy individuals. Such nature of food poisoning helps in differentiating the conditions of outbreaks of food-borne infectious diseases which generally spread over a period of several days. This standard covers the method for isolation and identification of *Shigella* responsible for food poisoning.

This Indian Standard was originally published in 1970 and first revised in 1976 when it was split into parts: Part III covering the methods for both *Salmonella* and *Shigella*. On a review by the technical committee responsible for formulating standards in the area of food microbiology, the Part III is being revised to separate the provisions. The provisions of *Salmonella* have been aligned with ISO 6579 : 1993 and retained in Part 3; whereas the provisions of *Shigella* have been covered in Part 7 of the standard.

In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (revised)'.

Indian Standard

METHODS FOR DETECTION OF BACTERIA RESPONSIBLE FOR FOOD POISONING

PART 7 GENERAL GUIDANCE ON METHODS FOR ISOLATION AND IDENTIFICATION OF *SHIGELLA*

1 SCOPE

This standard (Part 7) prescribes the method for isolation and identification of *Shigella*.

2 REFERENCES

The following Indian Standards contain provisions which through reference in this text, constitute provision of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

IS No.	Title
1070 : 1992	Reagent grade water (<i>third revision</i>)
5404 : 1984	Code of practice for handling of samples for microbiological analysis (<i>first revision</i>)
6850 : 1973	Agar, microbiological grade
6851 : 1973	Meat extract, microbiological grade
6852 : 1973	Bile salts, microbiological grade
6853 : 1973	Peptone, microbiological grade
7004 : 1973	Yeast extract, microbiological grade
7128 : 1973	Proteose peptone, microbiological grade
7590 : 1975	Gelatine, microbiological grade

3 SAMPLING

Samples shall be handled in accordance with the procedure given in IS 5404.

4 QUALITY OF REAGENTS

Unless specified otherwise, pure chemicals shall be employed in tests and distilled water (*see* IS 1070) shall be used where use of water as a reagent is intended.

5 GENERAL CHARACTERISTICS

Aerobic, Gram-negative rods, non-motile, catalase-

positive (exception in *Sh. dysenteriae* 1, that is, *Sh. shiga*), oxidase — negative, citrate — negative, shows fermentative reaction in Hugh-Leifson's test, ferment 'sugars' without gas production (exceptions are some strains of *Sh. flexneri* 6). The genus *Shigella* has been subdivided on the basis of biochemical characters and serological testing into four subgroups, subgroup A (*Sh. dysenteriae*, containing 10 serotypes, 1-10), subgroup B (*Sh. flexneri*, containing 6 serotypes, 1-6, and two variants, X and y), subgroup C (*Sh. boydii*, containing 15 serotypes, 1-15) and subgroup D (*Sh. sonnei*, containing 2 forms, phase I and II). The isolates belonging to the genus *Shigella* can be confused with those belonging to *Hafnia*, *Providencia*, *Aeromonas* and the anaerogenic, non-motile and non-lactose fermenting *Escherichia coli*. The useful biochemical characters which enable identification of a suspect strain as *Shigella* are given in Table 1.

Shigellae are much less reactive than *E. coli* and an isolate that produces acid promptly (that is within 24 hours) from a wide variety of carbohydrates, undoubtedly is not a *Shigella*. However, any organism which is biochemically a *Shigella* even if it does not agglutinate in the available typing sera, must be regarded as a *Shigella* and sent to a reference laboratory for confirmation and serotyping.

6 MEDIA

6.1 Nutrient Broth

Mix and dissolve by heating 10 g peptone (*see* IS 6853), 10 g meat extract (*see* IS 6851), and 5 g sodium chloride in 1 000 ml water. When cool, adjust pH to 7.5 to 7.6. Remove precipitate by filtration through filter paper. Sterilize by autoclaving at 120°C for 15 minutes.

6.1.1 Nutrient Agar

To the ingredients an in 6.1, add agar (*see* IS 6850) in such a concentration as will solidify and produce a sufficiently firm surface when poured in sterile petri dishes. The concentration of agar to be added varies from batch to batch and needs to be adjusted accordingly. Usual concentration required vary from 1.5 to 3 percent. Dissolve the agar in nutrient broth

and sterilize by autoclaving at 120°C for 15 minutes. Prepare plates and slopes as required.

6.2 Kauffmann-Muller's Tetrathionate Broth

To 90 ml of sterile nutrient broth (6.1), add with aseptic precautions and continuous agitation, 5 g calcium carbonate which has previously been powdered and sterilized in the autoclave, 10 ml of a 50 percent solution of sodium thiosulphate (previously sterilized by steaming), 2 ml potassium iodide/iodine solution (dissolve by grinding in a mortar 5 g of potassium iodide in 20 ml water and then add slowly with constant grinding 4 g iodine; keep the solution in dark glass-stoppered bottle), 1 ml of 0.1 percent aqueous solution of brilliant green and 5 ml of sterile ox bile or 1 g of bile salt (see IS 6852). Distribute in 10 ml quantities in sterile test tubes. The medium shall not be subjected to sterilization after preparation as the tetrathionate may decompose on heating.

6.3 Selenite F Broth

Dissolve 4 g sodium acid scienite, 5 g peptone (see IS 6853), 4 g lactose, 9.5 g disodium hydrogen phosphate and 0.5 g sodium dihydrogen phosphate in 1 000 ml of sterile water (water autoclaved at 120°C for 30 minutes) with sterile precautions and distribute the yellowish solution in 10 ml amounts in sterile screw-capped bottles. Steam at 100°C for 30 minutes. The medium shall not be autoclaved. There may be a slight red precipitate in the medium, but this does not interfere with the action of the medium. The pH of the medium as prepared shall be 7.1 and the quantity of phosphates added may be varied slightly to achieve this.

6.4 Desoxycholate Citrate Agar Medium

- a) *Agar Base* — Dissolve by heating 20 g meat extract (see IS 6851) in 200 ml water, and make the solution just alkaline to phenolphthalein with 50 percent sodium hydroxide solution. Boil and filter through filter paper. Adjust to pH 7.3, make up to 200 ml and add 20 g proteose peptone (see IS 7128). In another vessel, dissolve 90 g agar (see IS 6850) in 3 700 ml water by steaming for one hour. Filter the agar solution, add to it the meat extract-peptone solution and mix. Add 5 ml of 2 percent neutral red solution in 50 percent ethanol and 40 g lactose. Make up to 4 litres with water. Mix, bottle accurately in lots of 100 ml and sterilize in the autoclave by free steaming for one hour and then at 120°C for 10 minutes.
- b) *Solution A* — Dissolve 17 g sodium citrate, 17 g sodium thiosulphate and 2 g ferric

ammonium citrate (green scales) in 100 ml of sterile water, with heating.

- c) *Solution B* — Dissolve 10 g sodium desoxycholate in 100 ml of sterile water.
- d) Sterilize solutions A and B at 60°C in a water-bath for one hour. For preparing the desoxycholate citrate agar medium melt 100 ml of the agar base in a water-bath and add 5 ml each of the solutions A and B in the order given, using separate pipettes. Mix well after each addition. Cool the tube at 50 to 55°C. Pour into sterilized petri dishes (12 to 15 ml in each) and allow to set. Dry the surface of the medium in the incubator before use.

6.5 MacConkey Agar Medium

Mix 5 g sodium taurocholate or bile salts (see IS 6852), 20 g of peptone (IS 6853), 5 g sodium chloride and 15 to 30 g agar (see IS 6850), with 1 000 ml water. Steam until the solids are dissolved. Cool to about 50°C, and at this temperature adjust reaction to pH 7.6 to 7.8. Autoclave at 120°C for 15 min and filter while hot through a good grade of filter paper, or a plug of cotton wrapped in gauze and placed in the funnel. Adjust reaction of the filtrate to pH 7.3 at 50°C or pH 7.5 at room temperature. Add 100 ml of 10 percent aqueous solution of lactose (or 10 g lactose) and 3.5 ml of 2 percent solution of neutral red in 50 percent ethanol. Mix thoroughly, distribute into sterilized flasks and sterilize in the autoclave at 120°C for 15 minutes. For use, melt in the steamer, pour into sterile petri dishes and allow to set.

6.6 Nutrient Agar Medium for Motility Test

The medium consists of 3 g meat extract (see IS 6851), 10 g peptone (see IS 6853), 5 g sodium chloride, and 4 to 5 g agar (see IS 6850), dissolved in 1 000 ml water. Adjust the pH to 7.5 to 7.6. Put the dissolved medium into test tubes to fill a part of the tube and place into this a glass tube open at both ends. One end of the glass tubing shall project from above the surface of the agar for not less than 1.5 cm. Sterilize the tubes with the medium at 120°C for 15 minutes, and then cool. The consistency of the agar should be soft but not liquid. This is achieved by altering the amount of agar used, if necessary.

6.7 Medium for Hugh-Leifson's Test

Dissolve by heating in 1 000 ml water; 2 g peptone (see IS 6853); 5 g sodium chloride; 0.3 g dipotassium hydrogen phosphate; and 3 g agar (see IS 6850). Adjust pH to 7.1, filter and add bromothymol blue, 15 ml of 0.2 percent alcoholic solution. Sterilize at 115°C for 20 minutes. Add sterile solution of glucose

to give a final concentration of 1.0 percent. Mix and distribute in tubes, 14 mm × 100 mm, adding 3 to 4 ml per tube.

6.8 TSI Medium for H₂S Test

Heat to dissolve in 1 000 ml water, 3 g meat extract (*see* IS 6851), 3 g yeast extract (*see* IS 7004), 20 g peptone (*see* IS 6853), 1 g glucose, 10 g lactose, 10 g sucrose, 0.2 g ferrous sulphate (FeSO₄, 7H₂O), 5 g sodium chloride, 0.3 g sodium thiosulphate (Na₂S₂O₃, 5H₂O) and 15 to 30 g agar (*see* IS 6850); add 12 ml of 0.2 percent phenol red solution, mix and tube sterilizing by autoclaving at 115°C for 20 minutes. Pour into sterile test tube and cool to form a slope with deep butts.

6.9 Medium for Urease Test

Dissolve in 1 000 ml water, 1.5 g peptone (*see* IS 6853), 5 g sodium chloride, 15 to 30 g agar (*see* IS 6850), 2 g dipotassium hydrogen phosphate and add 6 ml of 1 : 500 aqueous solution of phenol red. Adjust pH to 6.8 to 6.9 and sterilize by autoclaving at 120°C for 15 minutes. When the solution has cooled to about 50°C, add a sterile solution of glucose to give a final concentration of 0.1 percent and add 100 ml of 20 percent solution of urea previously sterilized by Seitz filtration. Distribute in sterile test tubes to form deep slopes.

6.10 Simmon's Citrate Agar

Dissolve in 1 000 ml water, 5 g sodium chloride, 0.2 g magnesium sulphate (MgSO₄, 7H₂O), 1 g ammonium dihydrogen phosphate, 1 g dipotassium hydrogen phosphate, 2 g sodium citrate, and 15 to 30 g agar (*see* IS 6850); add 40 ml of 1 in 500 bromothymol blue indicator solution. Sterilize at 120°C for 15 min, tube and slant so that there is equal butt and slope.

6.11 Medium for Indole Production

The medium consists of 20 g peptone (*see* IS 6853) and 5 g sodium chloride in 1 000 ml water; adjust pH to 7.4. Place in tubes in 5 ml portion and sterilize at 120°C for 15 minutes. The medium should be tested with a strain of bacterium known to produce indole.

6.12 Peptone Water Medium for Carbohydrate Fermentation Tests

Dissolve 10 g peptone (*see* IS 6853) and 5 g sodium chloride in 900 ml water and adjust the pH at 7.1 to 7.3 so that after addition of 10 ml of Andrade's indicator the pH should be 7.5. Sterilize at 115°C for 20 minutes. Dissolve 10 g of the requisite sugar in 90 ml water and steam for 30 minutes or sterilize by filtration. With sterile precautions, add 90 ml of this sugar solution and 10 ml of Andrade's indicator solution to 900 ml of the sterile peptone water solution.

Distribute into sterile test tubes containing inverted Durham's fermentation tubes, and steam for 30 min. Andrade's indicator solution is prepared by adding 1 N sodium hydroxide solution to 0.5 percent aqueous solution of acid fuchsin until the colour of the indicator solution is just yellow.

6.13 Medium for Dihydrolase and Decarboxylase Activity

Dissolve with heat, 5 g peptone (*see* IS 6853), 5 g meat extract (*see* IS 6851), 5 mg pyridoxal, and 0.5 g glucose in 1 000 ml of water. Adjust pH to 6.0 and add 5 ml of 0.2 percent solution of bromocresol purple and 2.5 ml of 0.2 percent solution of cresol red. Sterilize in an autoclave at 115°C for 20 minutes. Divide this base medium into four equal volumes, and to each of the three add the respective amino acids (arginine, lysine and ornithine) as a final concentration of 1 percent if the *l*-form of the amino acid is used or as 2 percent if the *dl*-form of the amino acid is used. The fourth aliquot of the base medium does not contain amino acid and serves as control. Distribute the four media in 1 to 1.5 ml amounts into small sterilized tubes, 67 mm × 10 mm, and layer with sterile liquid paraffin to a height of about 5 mm. The pH of the final medium should be readjusted, if necessary, to 6.0. Sterilize in an autoclave at 115°C for 10 minutes.

6.13.1 An alternative medium where the base medium contains 5 g peptone (*see* IS 6853), 3 g yeast extract (*see* IS 7004), 1 g glucose and 10 ml of 0.2 percent bromocresol purple in 1 000 ml water may be used. The pH is adjusted to 6.7 and the remaining procedure for preparation of the medium is as given in 6.13.

6.14 Medium for Malonate Test

Dissolve by heating, if necessary, in 1 000 ml water, 1 g yeast extract (*see* IS 7004), 2 g ammonium sulphate (NH₄)₂SO₄, 0.6 g of dipotassium hydrogen phosphate, 0.4 g potassium dihydrogen phosphate, 2 g sodium chloride, 3 g sodium malonate, 0.25 g glucose and 0.025 g bromothymol blue. Dispense in about 3 ml portion into sterilized test tubes and autoclave at 120°C for 15 minutes. The final pH should be 6.7 ± 0.1.

6.15 Gelatin Liquefaction Test Medium

Dissolve in 1 000 ml water, 3 g meat extract (*see* IS 6851), 5 g peptone (*see* IS 6853), 120 g gelatin (*see* IS 7590) and 30 g sodium chloride. Distribute into sterilized test tubes and sterilize at 120°C for 12 minutes. The final pH should be 7.0.

7 PROCEDURE FOR ISOLATION

7.1 In general, all foods which have received dry heat treatment, namely, desiccation, dehydration or powdering, shall be pre-enriched in a non-inhibitory

medium such as nutrient broth (6.1). Raw foods and finished products which are suspected to be grossly contaminated after processing should not require pre-enrichment and the procedure as given in 7.1.1 shall be followed.

7.1.1 Where pre-enrichment is not required, the sample is blended in a sterile blender jar for 2 minutes or macerated with sterile sand in a sterile mortar using approximately 200 ml selenite F broth (6.3) per approximately 25 g of one portion of the sample, and 200 ml tetrathionate broth (6.2) per approximately 25 g of another portion of the sample. Incubate at 37°C for 24 hours. Raw meat samples should be incubated at 43°C, rather than at 37°C.

7.2 Isolation

7.2.1 From the incubated sample as in 7.1.1 (incubated at 37°C) plate out loopfuls on to desoxycholate citrate agar medium (6.4), and incubate for 24 hours at 37°C. It is also advisable to plate out on to the solid medium (6.4) loopfuls of each of the liquid medium after blending, but before incubating the plate at 37°C overnight as suspect *Shigella* colonies may be seen one day earlier.

7.2.2 If there is no suspect growth on the solid medium after 24 hours, reincubate for a further 24 hours. *Shigella* colonies on desoxycholate citrate agar shall be opaque, with a ground-glass appearance and with even margins.

8 TESTS FOR IDENTIFICATION

8.1 Pick out as many suspect colonies as are possible (not less than 5) to investigate and plate out into MacConkey agar plates (6.5) and incubate overnight at 37°C to make certain of the purity of the colonies. Pure colonies are transferred on to nutrient agar slopes (6.1.1) to obtain a heavy growth for further testing, as also for preserving the isolates for serotyping and/or phage typing.

8.2 *Shigella* are suspect when conforming to the characters given in Table 1.

8.2.1 Gram's Stain

The stain consists of: (a) 0.5 percent methyl violet or crystal violet in water, (b) iodine solution (1 percent iodine and 2 percent potassium iodide in water), and (c) counterstain (0.1 g neutral red, 0.2 ml of 1 percent acetic acid and 100 ml water).

On a clean grease-free slide, very light and thin smear, covering a small area, is made directly from liquid culture and in clean tap water from solid media. The smear is fixed by passing to and fro over a flame and cooled. Cover the smear with the stain (a) for 30 seconds, pour off the stain and wash with (b) and

then cover with (b) and allow to remain for 30 seconds. Wash off with ethanol until the dye ceases to stream out. Wash in running tap water and apply (c) for about one minute. Wash in tap water and dry for examination.

8.2.2 Test for Motility

Inoculate by stabbing with a straight wire into the top of the medium as in 6.6, the strain to be tested, inside the glass tubing to a depth of about 5 mm. Take care that inoculation is not made on to the surface of the medium outside the glass tubing. Incubate at 37°C for 18 to 24 hours. Motile strains shall be found to show growth on the surface of the medium outside the 'inner glass tube' having travelled through the entire medium inside this inner tube. If negative on the first day, keep the inoculated tube at room temperature for a further 4 to 6 days to see if evidence of motility is present.

8.2.3 Test for Catalase

Grow the strain for 24 hours on nutrient agar slope (6.1.1) and pour 1 ml hydrogen peroxide over the growth with the tube in a slanting position. Release of oxygen, shown as bubbles, from hydrogen peroxide indicates presence of catalase.

8.2.4 Test for Oxidase

To nutrient agar (6.1.1) slant of the culture, freshly grown, is added a few drops of freshly mixed test reagents, 1 percent solution of alpha-naphthol in 95 percent ethanol and equal amount of 1 percent solution of para-amino-dimethylaniline hydrochloride in water. A positive reaction is indicated by the appearance of a blue colour within two minutes.

8.2.5 Hugh-Leifson's Test

The strain from fresh nutrient agar growth (see 6.1.1) is stabbed into two tubes of medium (6.7), one of which is then layered over with a small amount of sterile liquid paraffin. Incubate both tubes at 37°C and observe up to 4 days. Acid formation, yellow colour, in the tube without paraffin indicates oxidative utilization of glucose. Acid in both tubes indicates fermentative reaction. Lack of acid in either tube indicates the strain as not being able to utilize glucose oxidatively or fermentatively.

8.2.6 Test for H₂S Production

Inoculate TSI medium (6.8) by stabbing the strain into the butt and streaking the slope. Incubate at 37°C and observe daily for up to 7 days. The presence or absence of blackening in the butt of the medium is to be recorded.

8.2.7 Test for Urease

Inoculate the organisms from 24-hour incubated

nutrient broth (6.1) culture heavily over the entire slope surface of medium as in 6.9 and incubate at 37°C for 18 to 24 hours. A positive urease is shown by the medium becoming pink or red on incubation. If negative, continue incubation for at least 4 days. *Proteus* species give a positive result, and can be used as 'control'.

8.2.8 Phenyl Pyruvic Acid Production

Obtain an overnight heavy growth of the organisms on nutrient agar (6.1.1). Suspend the growth in 0.5 ml of normal saline and transfer to a wide-bore test tube (diameter at least 1.5 cm). Add 0.5 ml of 0.2 percent *dl*-phenylalanine in normal saline. Mix and keep lying horizontally for at least three hours at room temperature. Add a few drops of half-saturated ferric chloride solution. A positive reaction is an immediate deep green colour which fades on keeping. *Proteus* strains give a positive reaction and serves as a control.

8.2.9 Test for Citrate Utilization

Inoculate the strain on to medium as in 6.10 with a young nutrient agar (6.1.1) slant culture using a straight wire. Incubate at 37° for up to 4 days for growth of the organisms.

8.2.10 Test for Indole

Inoculate medium (6.11) with a loopful of 24 hours growth in nutrient broth (6.1) and incubate at 37°C for 48 hours. Add 0.5 ml Kovac's reagent, prepared by dissolving 10 g para-dimethylaminobenzaldehyde in 150 ml amyl or *iso*-amyl alcohol and to which is added slowly 50 ml of concentrated hydrochloric acid. Prepare the reagent in small quantities and store in refrigerator. After adding Kovac's reagent, shake the tube gently, the appearance of a red colour indicates the presence of indole.

8.2.11 Tests for Fermentation Carbohydrates

Inoculate each of medium as in 6.12, and the carbohydrates indicated in Table 1 using a 1 percent concentration for the lactose, and incubate at 37°C for 18 to 48 hours. Record the presence of acid from pink colour and that of gas in the Durham's tube.

8.2.12 Test for Dihydrolase and Decarboxylase Activities

Inoculate, using a straight wire, through the liquid paraffin each of the four tubes of the medium as described in 6.13 from a freshly grown culture in nutrient agar (6.1.1). Incubate at 37°C and examine daily for up to 4 days only. The medium first becomes yellow due to acid production from the glucose; later, if dihydrolation or decarboxylation of the respective amino acid occurs, the medium becomes violet in colour.

Table 1 Useful Characters of *Shigella* Organisms

(Clauses 5.1, 8.2, 8.2.11 and 9.1)

Sl No.	Tests	Sub Gr. A	Sub Gr. B & C	Sub Gr. D
(1)	(2)	(3)	(4)	(5)
i)	Gram-negative rods	+	+	+
ii)	Motility	—	—	—
iii)	Catalase	+	+	+
iv)	Oxidase	—	—	—
v)	Hugh-Leifson's test	F	F	F
vi)	TSI for H ₂ S	—	—	—
vii)	Urease	—	—	—
viii)	Phenyl pyruvic acid	—	—	—
ix)	Citrate	—	—	—
x)	Indole	varies (b)	varies (b)	varies
xi)	Glucose	+	+	+
xii)	Lactose (1 percent)	—	—	(+)
xiii)	Sucrose	—	—	(+)
xiv)	Salicin	—	—	—
xv)	Dulcitol	— (c)	— (c)	—
xvi)	Mannitol	—	+	+
xvii)	Lysine	—	—	—
xviii)	Arginine	— or +	— or +	— or +
xix)	Ornithine	—	— (d)	+
xx)	Malonate	—	—	—
xxi)	Gelatin	—	—	—

NOTE — Some important exceptions to the above characters are as follows:

- (a) *Sh. dysenteriae* 1 (*Sh. shiga*) is catalase-positive;
- (b) *Sh. dysenteriae* 1 (*Sh. shiga*), *Sh. flexneri* 6 are always indole-negative, and *Sh. dysenteriae* 2 is always indole-positive; (c) Some strains of *Shigella* subgroup A, *Sh. flexneri* 6 and subgroup C are dulcitol-positive;
- (d) *Sh. boydii* 13 is ornithine-decarboxylase-positive.

+ = Present or Positive

(+) = Late Positive

— = Negative or no reaction

F = Fermentative reaction

When performing the test, for each amino acid always use strain(s) of bacterium (a) known to show positive result and strain(s) of bacterium (a) known to show negative result. These serve as 'controls'.

8.2.13 Test for Utilization of Malonate

Inoculate the medium as in 6.14 and incubate for 24 hours at 37°C. A positive malonate reaction is indicated by a deep blue colour and a negative reaction by the unchanged greenish colour of the medium. With

some negative strains, a yellow colour appears.

8.2.14 Gelatin Liquefaction Test

Inoculate the strain into medium as in 6.15 as a 'stab culture' using a straight wire and incubate at 22°C for 4 days in an upright position. In warm temperatures, the medium is usually in a semi-solid state. Before inoculation, and also for reading the result for liquefaction, the tubes should be kept in an ice-bath or refrigerator to facilitate making a stab culture, and also taking the reading.

9 SEROTYPING

Shigella strains are named on the basis of serotyping. Polyvalent *Shigella* sera, if available, will help in confirming strains as being shigellae. Strains conforming to the morphological characters and biochemical reactions in Table 1 shall be sent to reference laboratories for confirmation and final identification.

NOTE — At present the Department of Pathology and Bacteriology, K.G. Medical College, Lucknow (U. P.) serves as National *Shigella* Centre.

Bureau of Indian Standards

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Review of Indian Standards

Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Handbook' and 'Standards : Monthly Additions'.

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